there was no purification over the initial whole homogenate.

Cells grown anaerobically in the presence of 10% (w/v) glucose contained no detectable cytochrome oxidase or catalase. Cells grown anaerobically with 5% (w/v) glucose (and maintained as anaerobic as possible during sphaeroplast production and lysis) did, however, show low activities of both these enzymes. The percentage sedimentability of enzymes in homogenates of these cells was as follows: protein, 7%; acid phosphatase and esterase, not sedimentable; NADH-cytochrome *c* oxidoreductase, 19%; NADPH-cytochrome *c* oxidoreductase, 4%; NADPH-ferri-cyanide reductase, 33%.

Cells grown anaerobically with galactose in the presence of Tween 80 and ergosterol (Wallace, Huang & Linnane, 1968) do possess detectable activities of cytochrome oxidase and catalase and the percentage sedimentability of these enzymes was 87% and 55% respectively. The median density of sedimentable protein (1.19) corresponded with that of NADH- and NADPH-cytochrome *c* oxidoreductases and NADH- and NADPH-ferri-cyanide oxidoreductases; acid phosphatase was 47% sedimentable.

R.B. was I.C.I. Fellow during the course of this work.

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The Subcellular Acquisition of Carbohydrates by Glycoproteins

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Several reports have indicated that the microsomal membranes are of primary importance in the addition of carbohydrate to the protein moiety of glycoproteins (Lawford & Schachter, 1966; Molnar, Robinson & Winzler, 1965). We have investigated the subcellular sites of acquisition of carbohydrate by protein in the immunoglobulin A-secreting plasmacytoma, MPC-1, by using a technique of incubation *in vitro*.

Homogenization and fractionation of incubated myeloma tissue on a continuous sucrose gradient gives a number of distinct bands. The specific radioactivity of protein from these bands after incubation of the tissue with [¹⁴C]lysine is largely uniform, whereas there are marked differences between the specific radioactivities of bands obtained after incubation with [¹⁴C]glucosamine. Uptake of both [¹⁴C]lysine and [¹⁴C]glucosamine is linear with time up to 140 min. The radioactive myeloma protein synthesized in these subcellular

fractions was identified by immunoelectrophoresis of sonicated extracts of the fractions, followed by radioautography of the resulting precipitin lines.

In the presence of puromycin at a concentration that inhibits protein synthesis by 98%, [¹⁴C]-glucosamine incorporation into trichloroacetic acid-insoluble glycoprotein is decreased by only 50–60%. Further, there is a decrease of only 20–30% in radioactive glycoprotein appearing in the incubation medium. Thus there must exist in the cell a large pool of partially glycosylated protein that continues to be glycosylated and secreted after protein synthesis has virtually ceased.

Whether ribosomes are involved in the initial attachment of hexosamine to glycoprotein is a matter of contention. Preparations of ribosomes from tissue incubated with [¹⁴C]glucosamine showed a linear increase in specific radioactivity up to 140 min. Tissue preincubated with [¹⁴C]glucosamine for 30 min. and 70 min., and subsequently incubated for a further 10 min. with added puromycin, showed an exactly similar decrease in the specific radioactivity of the ribosomes after the puromycin addition. However, this decrease constituted only 29% and 16% respectively of the total specific radioactivity of the ribosomes. Thus it is suggested that two factors contribute to ribosomal labelling: glucosamine attached to nascent polypeptides, and radioactivity from adsorbed labelled material.

N.J.C. is indebted to the Medical Research Council for a scholarship for training in research methods.

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Studies on Sialoglycoprotein Complexes of Human Erythrocyte Membranes

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Proteins and glycoproteins have been prepared by the disruption of erythrocyte membranes in several ways (Rega, Weed, Reed, Berg & Rothstein, 1967; Blumenfeld, 1968; Harris, 1968; Marchesi & Steers, 1968; Rosenberg & Guidotti, 1968; Schneiderman & Junga, 1968). We have modified the techniques of Marchesi & Steers (1968) and Blumenfeld (1968) to enable us to prepare two glycoprotein complexes from human erythrocyte 'ghosts'.

Erythrocyte 'ghosts' (water-washed, 2%, w/w) were treated with mercaptoethanol (50 mM) and

ATP (3mm) at 4°. After centrifugation at 76 900g, the supernatant was concentrated and freeze-dried (complex I). The pellet was dissolved in aq. 33% (v/v) pyridine at 0°, diluted and dialysed against water. After centrifugation at 76 900g, the supernatant was concentrated and freeze-dried (complex II). Complex I contained 0.2% (w/w) of sialic acid; complex II contained 10% (w/w) of sialic acid and readily gelled.

Zone electrophoresis of the complexes on polyacrylamide at pH 2.2 (Takayama, MacLennan, Tzagoloff & Stoner, 1966) gave a pattern of about 16 zones. Excision and re-running of the zones gave no further resolution and the patterns were unaffected by the freeze-drying of the materials. Both complexes gave similar patterns and there were no differences between batches. Carbohydrate was localized in slow-moving zones and aggregated material in complex II, as was material that bound Ruthenium Red.

Complex II was degraded by alkali above pH 9.0 at 20° and at pH 10.5–11.0 at 0°. Neuraminidase removed certain components of low electrophoretic mobility from it and it was selectively degraded by trypsin in the presence of 10mM-Ca²⁺. Treatment with 0.1% (w/v) sodium dodecyl sulphate for 12hr. at 4° modified the electrophoretic pattern of complex II. The aggregation of the complex was examined in the analytical ultracentrifuge.

Many of the electrophoretic zones represent distinct species or mixtures of species of proteins, and the glycoproteins form a distinct group among these. Sialic acid may be involved in the aggregation of complex II.

We thank Dr J. C. Metcalfe for discussion.

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The Effect of Intraductal Prolactin on Protein and Nucleic Acid Biosynthesis in the Rabbit Mammary Gland

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In a recent report (Falconer & Fiddler, 1968) it was suggested that a stimulation of RNA and protein biosynthesis was required for the increase in activity of the enzyme lipoprotein lipase that occurred during a period of prolactin-induced lactogenesis. The aim of this work was therefore to verify or otherwise this suggestion.

Pseudo-pregnancy was induced in virgin oestrous rabbits by the intravenous injection of 50 i.u. of chorionic gonadotrophin, and lactation was initiated by the intraductal injection of 50 µg. of prolactin (N.I.H.-P-S8)/duct. In these experiments individual glands were injected with prolactin at regular intervals. On the third or fourth day after the first injection, [¹⁴C]leucine (0.1 µC in 0.1 ml. of 0.9% NaCl/duct) was intraductally injected into the prolactin-treated glands and also into a control gland. All the glands were removed after 1 hr. for determination of the specific radioactivity of total protein (Mayne, Barry & Rivera, 1966).

Parallel experiments that determined the incorporation of [³H]uridine into RNA and [¹⁴C]-thymidine into DNA were carried out. The labelled precursors were injected either simultaneously (0.2 µC of [³H]uridine and 0.1 µC of [¹⁴C]thymidine in 0.1 ml. of 0.9% NaCl/duct) or separately in different animals. The isolation of DNA and RNA was essentially by the method of Munro & Fleck (1966) with slight modification.

The response to prolactin measured by [¹⁴C]-leucine incorporation into protein was characterized by two marked peaks in activity. The specific radioactivity increased dramatically during the first 24 hr. after injection of prolactin, then fell to near basal value during the next 24 hr. and subsequently increased again to form a second peak of incorporation 72 hr. after injection of prolactin, and remained at an elevated value at 96 hr.

The patterns of [³H]uridine and [¹⁴C]thymidine incorporation into RNA and DNA respectively were similar, and showed two marked peaks preceding in time the peaks in protein biosynthesis. An initial peak of incorporation of bases into both nucleic acids was observed during the first 12 hr. after injection of prolactin, this subsequently decreasing to the initial value during the next 12 hr. and then increasing again to form a second peak of activity 30–36 hr. after the hormonal stimulation. At 48 and 60 hr. after injection of prolactin much less incorporation was observed than at 30 hr.